See Colour Plates 109, 110.

See also: **II/Chromatography: Liquid:** Mechanisms: Size Exclusion Chromatography. **III/Bitumens: Liquid Chromatography. Crude Oil: Liquid Chromatography. Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Geochemical Analysis: Gas Chromatography. Polycyclic Aromatic Hydrocarbons:** Gas Chromatography; Solid-Phase Extraction; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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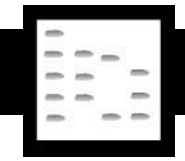
PHARMACEUTICALS



Basic Drugs: Liquid Chromatography

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Introduction

High performance liquid chromatography (HPLC) is the most important technique for the separation, analysis and quantification of a wide range of drug types. Although there are a variety of approaches available for the chromatography of basic drugs, analysis of these compounds is still one of the main challenges for the practising chromatographer in the pharmaceutical industry. The general approaches have remained the same since the early days of HPLC, but there have been many refinements and developments since the late 1960s. In the main these have involved modification and improvements to the stationary phase, which are still continuing today.

This article focusses on the main methods of separation and analysis of basic drugs that are currently in use. Consideration is given to the relative pros and cons of the different approaches, as well as the development and evolution of the techniques.

Liquid–Solid Chromatography

Liquid–solid, or normal-phase chromatography (LSC) was one of the first approaches employed for the separation of bases in modern LC. Its use, however, has decreased dramatically since the 1970s and it is now rarely employed for the routine separation of basic drug molecules.

LSC was originally carried out using native silica or alumina, with the former being preferred for the separation of bases. Recently, there has been a gradual shift towards the use of polar bonded phases such as cyanopropyl, amino or diol, the last two showing preferential retention of bases compared with cyanopropyl. These bonded materials overcome some of the problems associated with silica phases such as deactivation by water and long equilibration times. The problem of deactivation is particularly acute in the area of bioanalysis, where it can be difficult to obtain extracts that are totally dry. To a certain degree this problem can be overcome by the inclusion in the eluent of a small amount (1% v/v) of water or a short-chain alcohol.

Eluents for LSC typically consist of mixtures of a nonpolar hydrocarbon, such as hexane or isooctane, and a polar modifier, e.g. dichloromethane, 2-propanol, methyl *t*-butyl ether or ethyl acetate. Frequently, the addition of an amine modifier such as triethylamine may be necessary to give satisfactory peak shapes.

LSC does complement reversed-phase separations, in that the selectivity is very different, with the order of elution usually being reversed. Unlike the more widely used reversed-phase HPLC (RP-HPLC; see below), it is particularly suited to the separation of geometric isomers.

Reversed-phase HPLC

RP-HPLC for pharmaceutical analysis took off in the early 1970s with the introduction of commercially available microparticulate bonded packings. Although it rapidly became the dominant mode of chromatography in the pharmaceutical area, it quickly became apparent that the chromatography of basic compounds was not a straightforward matter. Despite this, RP-HPLC still figures prominently in both literature and pharmacopeia methods.

The analysis of basic solutes using RP-HPLC methods presents a number of problems, principally because the analyte is retained by a number of retention mechanisms (some of which are poorly understood) in addition to the expected hydrophobic interaction. These include: hydrogen bonding, π–π interactions, ion exchange, ion pair formation and salting out. It is the multiplicity of these retention mechanisms that often leads to poor chromatographic performance, characterized by low peak efficiencies, tailing or asymmetric peaks and retention times that are dependent on the mass of compound injected.

Many of these phenomena can be traced back to the presence of unreacted silanols on the bonded silica surface. Despite the use of forcing conditions during the bonding process, there always remains a significant number of unreacted silanols, around 60% of the total. Silanols are acidic and if ionized can function as ion exchange sites. They are also polar and are able to interact with solutes via hydrogen bonding. While hydrogen bonding is not considered to be a serious problem, ion exchange can be particularly troublesome. From an energetics point of view this is easy to understand since the energy involved in coulombic or ionic interactions is around 20 times greater than that of hydrophobic interactions. Much of the development of RP-HPLC phases has been directed at minimizing the number (or type) of silanols, or at least minimizing their influence.

The interaction of a basic solute with the residual silanols is dependent on the pK_a of the base and the stereochemistry around the basic centre. The higher the pK_a and the lower the steric hindrance around the basic centre, the greater the interaction with silanols. Thus pyridine, which is a relatively weak base (pK_a 5.25), is also probably the smallest and least hindered aromatic base and can be particularly difficult to chromatograph on reversed-phase materials.

A number of general strategies involving both changes to the mobile phase and the stationary phase have been employed to improve the chromatography of bases. Each of these is discussed below.

Stationary Phase Modifications

End-capping

For many years manufacturers attempted to eliminate the residual silanols through a process known as end-capping. Following the primary bonding procedure, the stationary phase is further reacted with a small silylating reagent such as trimethylchlorosilane (TMCS) or hexamethyldisilazane, both of which generate trimethylsilyl groups ($(CH_3)_3Si$–). The rationale for their use is that the small size of the trimethylsilyl group should allow access to the silanols, which are inaccessible to bulkier primary reagents such as octadecyldimethylchlorosilane. However, this approach has never been fully successful, and even after exhaustive end-capping, unreacted silanols always remain. It is also believed that some silanols are so reactive that although they can be end-capped, they rapidly hydrolyse in aqueous organic mobile phases (especially at acid pH) to regenerate the silanol.

Polymer-coated Phases

In this approach a polymer coat is formed over the surface of the silica. If desired this can be further modified by the addition of C_{18} groups, for example. Although partially successful in blocking access to the silanols, this approach also leads to reduced efficiency, presumably through poor mass transfer caused by blocking of the silica pores. Although packings based on this approach are commercially available, they are not widely used.

Sterically Protected Phases

Another approach to minimizing silanol interactions is through the use of sterically hindered silylating reagents, such as diisopropyloctadecylchlorosilane. The rationale here is that the bulky isopropyl groups (in contrast to the more commonly employed methyl groups) should result in steric occlusion of any residual silanols. This approach has only been partially successful in reducing analyte–silanol interactions and has not been widely adopted. However, it would appear to be more successful in stabilizing the bonded phase and allowing the use of a wider range of pH.

Base-deactivated Phases

In the mid-1980s it was demonstrated that it was the type, rather than the number of silanols that was responsible for the secondary interactions. A poor silica is characterized by an uneven distribution of silanols with varying and strong acidity – so-called Type A materials. In contrast, good silicas (Type B) are characterized by a large population of hydrogen-bonded, low-acidity silanols.

This discovery led to the development of the so-called base-deactivated phases, which were claimed to be superior to standard materials for the separation and analysis of bases. **Figure 1** presents three chromatograms showing the separation of a series of basic drugs using three reversed-phase packings. The first is a standard C_{18} material, the second is partially deactivated (typical of end-capped materials), and the third is a modern base-deactivated material. The improvement in peak shape and efficiency across these three types is clearly evident.

The purity of the silica that is used to make the bonded-phase material is also considered to be important in the analysis of basic drugs. Silicas with high purity and very low metal content, particularly aluminium and iron, have been shown to give improved performance. These metallic impurities can act in one of two ways: directly as centres for chelation, or indirectly through their polarization of silanols, thereby increasing their acidity or activity.

Much of the technology that has gone into the development of modern reversed-phase materials is proprietary. However, most of these stationary phases are probably made by employing one or several of the following approaches: fully rehydroxylated silicas with an even distribution of low acidity silanols; high purity silicas; sterically hindered silylating reagents; or more recently silylating reagents incorporating a polar linkage (e.g. carbamates). It is important to note that a column that gives particularly good performance with one compound may be totally unsuited to the analysis of another compound. This is clearly seen from the data in **Table 1**, which compares the performance of four commercial columns based on the tailing observed for a series of basic drugs. All the columns were claimed to be suitable for the analysis of bases. For example, column I is clearly the worst with atenolol and chlorpheniramine, giving rise to the most severe tailing peaks. With amiloride, however, column I is almost as good as column IV, the best, and column II stands out as being quite different. With pyridine as a simple test marker, all the columns give a similar poor performance. Before selecting a column, therefore, it is

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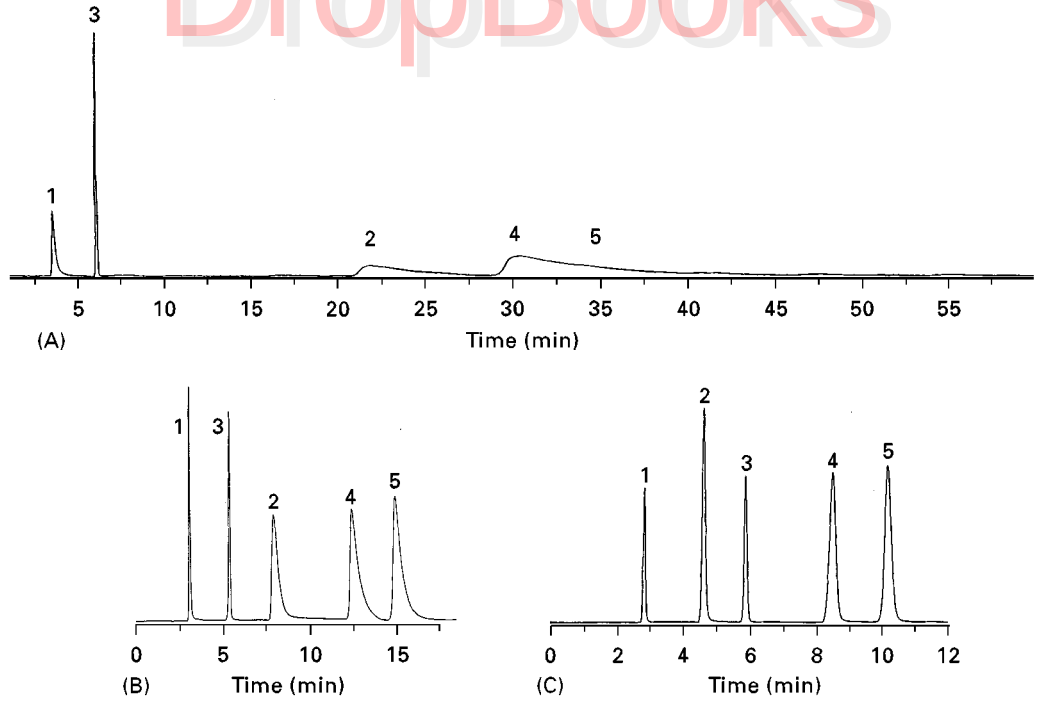

Figure 1 A comparison of the chromatography of a range of basic compounds on three C_{18} reversed-phase packings: (A) a conventional material, (B) a semi-deactivated material and (C) base-deactivated material. Eluent: methanol/KH_2PO_4 (25 mmol L^{-1}, pH 6.0) (80/20). Flow rate: 1 mL min^{-1}. Identification: 1, norephedrine; 2, nortriptyline; 3, toluene (neutral test marker); 4, imipramine; 5, amitriptyline. (Reproduced with permission from Hichrom, Reading, UK.)

Table 1 The tailing factor for a range of basic compounds on four base-deactivated reversed-phase columns

Compound	*Tailing factor on different columns*			
	I	*II*	*III*	*IV*
Atenolol	2.6	1.7	1.3	1.2
Pyridine	2.2	2.5	2.1	2.7
Pindolol	1.7	2.2	1.4	1.3
Amiloride	1.2	1.7	1.2	1.1
Cycolguanil	2.2	1.9	1.4	1.2
Chlorpheniramine	6.8	3.1	3.1	1.6

Eluent methanol/water, containing ammonium acetate (0.1 mol L^{-1}), pH approximately 7.1. (Reproduced from Law *et al.*, 1998, with permission from Elsevier Science.)

important to try it out with the given analyte using a variety of conditions, since some columns seem to perform better with acidic eluents while others work best at neutral pH. The use of a number of parameters such as retention, efficiency and peak tailing are necessary if a true measure of column performance with regard to a particular analyte is to be gained.

Most manufacturers and distributors of HPLC materials supply base-deactivated materials.

Polymeric Phases

In an attempt to eliminate the effects of silanols totally some manufacturers moved away from silica completely and developed polymeric phases based on styrene–divinylbenzene. These materials are claimed to offer pure reversed-phase chromatography devoid of the secondary interactions that so bedevil chromatography on silica-based materials. They also offer a significant advantage in that they are fully stable over the pH range 1–14. This allows chromatography of basic drugs in the unionized form at high pH, which is impossible with standard silica-based materials.

These materials, however, were not without disadvantages. Some of the early materials underwent swelling when moving from high aqueous to high organic eluents, resulting in back-pressure changes and column blocking. Surprisingly, however, they also suffered from secondary interactions that resulted in poor performance. It has been claimed that the grafting of alkyl chains (typically C_{18}) onto the polymer phase is a useful method of minimizing these undesirable interactions. The major disadvantage of these materials for most analytes, including basic drugs, is the poor peak efficiencies attributed to slow mass transfer. These materials have not found widespread general use, although they are used for some particular applications, such as the analysis of quaternary amines, where their high retentivity compared with comparable silica phases is particularly useful.

Eluent Modifications

Organic Modifier

It is a generally observed phenomenon that of the three commonly used organic modifiers – methanol, acetonitrile and tetrahydrofuran – the first gives the most symmetrical peaks with basic compounds. This is usually explained by the fact that methanol has both hydrogen bond acceptor and donor properties, in contrast to the other two solvents, which have only acceptor properties. Thus, methanol is able to interact and effectively block residual silanols to a greater extent.

Eluent Buffers

Phosphate buffers are commonly used in HPLC eluents, especially for reversed-phase work. Of the two counterions mainly used, potassium has greater affinity for the silanol ion exchange sites than sodium. Thus the use of potassium phosphate is recommended, since this will result in a small but significant improvement in peak shape. It follows also that the use of stronger buffers (100 mmol L^{-1}) should be more effective than buffers of lower concentration.

Eluent pH

The eluent pH can also have a marked effect on peak shape. Since the undesirable interactions are ionic, then an improvement in chromatographic performance should be achieved by suppressing the ionization of either the base or the silanol. Because many basic compounds of pharmaceutical interest are relatively strong bases ($pK_a > 8.5$), an eluent pH of around 10.5 would be required to suppress their ionization. Since many silica-based stationary phases undergo dissolution at pH > 7.5–8, this approach is impractical. More commonly, therefore, the eluent pH is reduced to around 2 to 3 through the addition of trifluoroacetic acid or phosphoric acid in an attempt to suppress the ionization of the silanols. This approach is not always fully successful because a small population of silanols are often quite acidic with pK_a values < 1. Acidified eluents, however, do complement the use of silanol-blocking and ion pair reagents (see below).

Silanol-blocking Agents

Silanol-blocking agents, which normally take the form of a lipophilic amine, can be considered as a special type of eluent additive. They are usually

included in the eluent at a concentration of around 25 mmol L^{-1}. If secondary or tertiary amines are used, then the eluent pH needs to be adjusted to ensure full ionization of the amine. Where quaternary amines are used the eluent can be at any pH, although a slightly acidic eluent would be recommended to minimize the number of ionized silanols.

The large excess of the protonated amine in the eluent effectively blocks or masks the residual silanols, making them less accessible to interaction with the basic analyte. A number of systematic studies have been carried out on the use of silanol-blocking agents. From these a number of general conclusions can be drawn. First, the silanol-blocking properties of alkylamines decreases in order primary < secondary < tertiary ≤ quaternary. Compounds of the type $(CH_3)_3N^+R$ or $(CH_3)_2N^+HR$, where R is a long alkyl chain, are the most effective. There is no consensus as to the optimal length of the alkyl chains; some workers recommend triethylamine (30–50 mmol L^{-1}) while others prefer dimethyloctylamine (5–10 mmol L^{-1}). As well as improving peak shape and efficiency, the use of masking agents can also lead to a significant reduction in retention for basic solutes. Silanol-blocking agents can also be used with base-deactivated materials, where significant improvements can be observed with some basic drugs.

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Dynamically Modified Silica

Introduced by Hansen in the early 1980s, this approach involves the creation of a dynamic reversed phase. A bare silica column is used with an aqueous/organic eluent containing a long-chain quaternary ammonium compound. Initially the eluent is passed through the column until equilibrium is reached, and the quaternary ammonium compound forms an adsorbed monolayer on the silica surface. This approach has been shown to give good peak shapes for strong bases (**Figure 2**) and to give separations difficult to achieve using base-deactivated materials. A major and somewhat surprising attribute of the system is the excellent reproducibility in selectivity, even when changing from one brand of silica to another. Thus this approach is particularly suited to those assays where long-term reproducibility is essential, such as in pharmaceutical quality control. Despite these advantages this method has not become widely used, possibly due to the limitations with respect to UV detection, particularly at short wavelength. The lack of compatibility with mass spectrometry, currently the favoured means of detection in the pharmaceutical industry, probably means that it will never become popular.

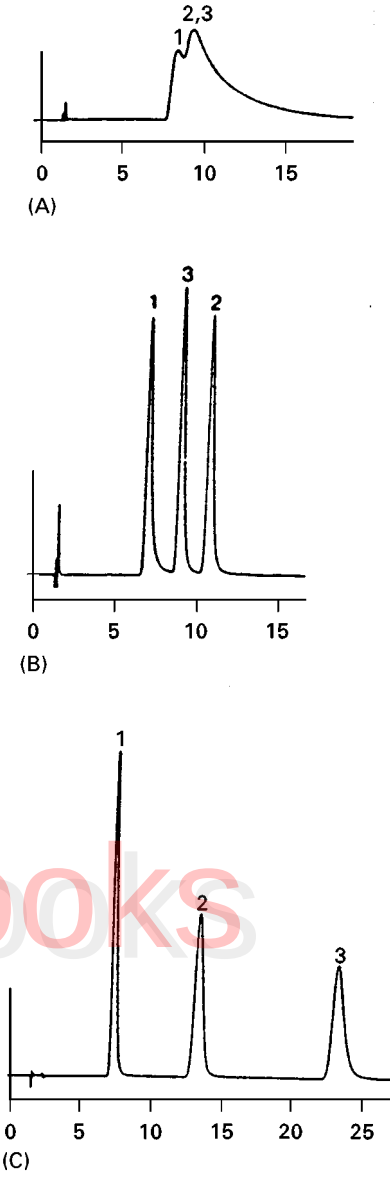

Figure 2 The separation of imipramine and metabolites under three sets of conditions. (A) Column, LiChrosorb RP-18 (120 mm × 4.6 mm); eluent, methanol/water/phosphate buffer (0.2 mol L^{-1}, pH 4) (60 : 35 : 5). (B) As for (A) but with the addition of dodecyltrimethylammonium bromide (2.5 mmol L^{-1}) and sodium dodecanesulfonate (5 mmol L^{-1}). (C) Column, LiChrosorb Si 60 (120 mm × 4.6 mm); eluent, methanol/water/phosphate buffer (0.2 mol L^{-1}, pH 7) (55 : 40 : 5) with the addition of cetytrimethylammonium bromide (2.5 mmol L^{-1}). Identification: 1, imipramine-*N*-oxide; 2, desipramine; 3, imipramine. (Reproduced from Hansen SH, Helboe P and Thomsen M (1987) Separation of basic drug substances by reversed-phase high-performance liquid chromatography on dynamically modified silica and on bonded-phase materials. *Journal of Chromatography* 409: 71–80, with permission from Elsevier Science.)

Ion Pairing HPLC (IP-HPLC)

This is an approach developed during the early 1980s and pioneered by Schill and co-workers. It is parti-

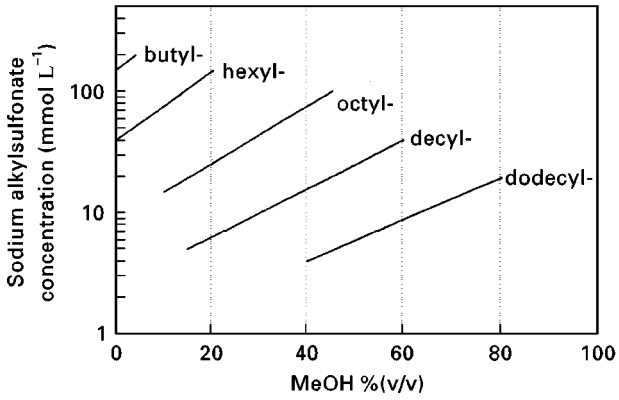

Figure 3 Recommended maximum mobile-phase concentrations of alkyl sulfonate pairing ions and their application range as a function of the mobile-phase methanol concentration. (Reproduced from Bartha A, Vigh G and Vorga-Puchony Z (1990) Basis for the rational selection of the hydrophobicity and concentration of the ion-pairing reagent in reversed-phase ion-pair high-performance liquid chromatography. *Journal of Chromatography* 499: 423–434, with permission from Elsevier Science.)

cularly useful for the separation and analysis of polar bases such as drugs containing quaternary amine groups, which are usually difficult to analyse under standard reversed-phase conditions because their high polarity leads to poor retention. Because of the general improvement in the quality of reversed-phase materials (end-capped, base-deactivated) the use of IP-HPLC has declined somewhat. Typical ion pair reagents are perchlorate or more commonly alkyl sulfonates (e.g. pentane, heptane or dodecane). The organic ion pair reagents are normally added at concentrations of around 10 to 100 mmol L^{-1} and, to ensure full ionization of the basic drug, the eluent is normally acidified to a pH of around 2–3. The longer the alkyl chain of the pairing ion, the greater the retention. Excellent control of selectivity with respect to other bases, as well as neutral or acidic compounds, is possible. This flexibility can also make optimization of the system complex, since as well as organic modifier type, modifier concentration and eluent pH, the nature and concentration of the ion pair reagent, as well as the concentration of the buffer, all have an effect on the separation. The optimization of such systems have been extensively studied. **Figure 3** shows how the nature and concentration of the pairing ion are dictated by the methanol concentration in the eluent.

In a number of applications, a quaternary ammonium ion has also been added to the eluent to further improve the peak shape of the basic analytes.

Ion Exchange HPLC (IE-HPLC)

Ion exchange is a useful, if under utilized, mode of chromatographing basic solutes. With this form of chromatography the ionic interactions, which prove so troublesome in RP-HPLC, are actually employed as the major retention mechanism. Two general approaches are available, the first employing native silica as the stationary phase and the second utilizing bonded phases bearing a specific ion exchange group.

Ion Exchange Using Native Silica

As stated earlier, the silanol groups on the surface of silica gel are weakly acidic with a bulk pK_a of around 4. Consequently at pH values greater than 4 the silanols become ionized and they are able to act as ion exchange sites. Silica is typically used with eluents consisting of a mixture of an organic modifier (methanol or acetonitrile) and an aqueous buffer at a pH > 7. A variety of buffers have been employed, but ammonium acetate is particularly useful since it has low UV absorbance, high volatility and is noncorrosive. A good general purpose eluent is methanol/ammonium acetate buffer (9 : 1 v/v, pH 9.1). When employed with Spherisorb S5W silica this system shows excellent performance for the analysis of strongly basic drugs with pK_a values > 8. Most bases, including some quaternary ammonium compounds, give excellent peak shapes (< 1.2) asymmetry and high efficiencies ($N = 50\,000$ plates m^{-1}). Despite the use of a high eluent pH the silica stationary phase shows excellent stability and the columns can be used for many months with no loss in performance. This unexpected stability of the silica is attributed to the use of a high proportion of organic modifier in the eluent and ammonia as the base, rather than sodium, potassium or a strong organic base, which are generally more aggressive.

This approach is particularly good for drug screening since a wide range of drug types elute in a relatively narrow retention range (**Figure 4**). Thus gradient elution, which would be needed to chromatograph a similar set of compounds under RP-HPLC conditions, is avoided. Retention is found to be dependent on the solute pK_a with the more basic compounds being more highly retained. Since the pK_a of basic drugs often does not change radically when the drug is metabolized, the system is useful for the analysis of metabolites in biological fluids. To a certain degree the retention of the metabolites is predicable, with most metabolites eluting after the parent compound in contrast to RP-HPLC.

The major limitation of this approach is its restriction to the analysis of strong bases with pK_a values > 8. The column-to-column reproducibility can also be a problem as 'ageing' of the silica, whether in the dry state or packed into a wet column, can result in significant selectivity differences.

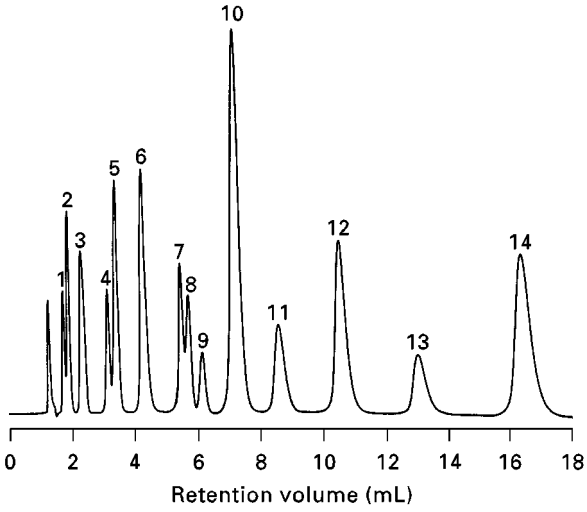

Figure 4 The separation of a range of bases using a Spherisorb S5W column (100 mm × 4.6 mm) and an eluent consisting of methanol/ammonium acetate buffer (9 : 1, v/v) with an apparent pH of 9.1. Identification: 1, tetracaine; 2, tamoxifen; 3, diphenhydramine; 4, amiloride; 5, thioridazine; 6, chlorpheniramine; 7, *N*-methylamphetamine; 8, debrisoquine; 9, 4-hydroxydebrisoquine; 10, quinacrine; 11, strychnine; 12, betahistine; 13 benzethonium; 14, pyrantel. (Reproduced from Law, 1990.)

Ion Exchange Using Bonded Phases

Although introduced not long after hydrocarbon-bonded phases, these materials have never really caught on for the separation of drugs. To a degree this may be due to the perceived complexity of ion exchange as a mode of separation, with the need to control the ionization of both analyte and stationary phase. However, the use of strong cation exchanger (SCX) materials, such as propylsulfonic acid, which are highly acidic ($pK_a < 1$) and effectively ionized at all pH values, makes the development of separations relatively straightforward.

Recently there has been renewed interest in bonded phase ion exchange materials with a number of reports appearing on applications of the propylsulfonic acid phases. To ensure full ionization of both weak and strong bases, acidic eluents should be employed, and to give good efficiencies and good selectivity, a high organic modifier concentration is recommended. Using a Spherisorb 5 μm SCX column with an eluent consisting of methanol/water/trifluoroacetic acid (800 : 200 : 2.3 v/v) containing ammonium formate (20 mmol L^{-1}) it has proved possible to chromatograph a very wide range of basic drugs. The system shows acceptable retention for strong bases such as the β-blockers ($pK_a \sim 9.5$) through to weak bases such as diazepam with a pK_a of only 3.4 (**Figure 5**). Like the silica-based system described above, this approach also gives very good performance with high efficiencies (up to 70 000 plates m^{-1}) and good peak symmetries. The retention is controlled in the main by the concentration of the ammonium formate buffer. Furthermore, through systematic modification of the eluent pH or organic modifier concentration, it is possible to change selectivity in a predicable manner.

One advantage of both the above ion exchange approaches is that the eluent constituents are relatively volatile ensuring compatibility with mass spectrometric detection.

Future Developments

RP-HPLC, utilizing silica-based materials, is likely to continue as the dominant technique for the analysis of pharmaceuticals including basic drugs. Despite their problems and limitations, silica-based materials are still able to outperform polymeric phases. Although the development of polymer-based materials is likely to continue, our knowledge and understanding of silica – despite its use in HPLC for over 30 years – is still growing. A number of academic groups and chromatography companies are actively researching silica. Although major breakthroughs in the

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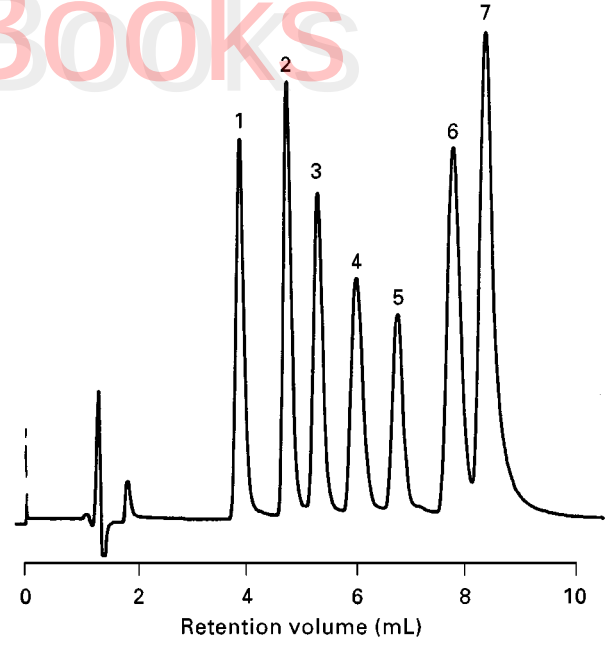

Figure 5 The separation of a range of basic drugs using a Spherisorb 5SCX column (100 mm × 4.6 mm) and an eluent consisting of methanol/water/trifluoroacetic acid (TFA) (800 : 200 : 2.3, v/v) containing ammonium formate (0.02 mol L^{-1}), with an apparent pH of 2.45. Identification: 1, halofantrine; 2, minoxidil; 3, haloperidol; 4, reserpine; 5, cimetidine; 6, verapamil; 7, clomipramine. (Reproduced from Law and Appleby, 1996.)

methods of production and quality of silica are unlikely, a process of refinement can be expected, leading to even better deactivated materials.

While it would be encouraging to believe that the benefits of some of the other approaches will be recognized and exploited, the conservative nature of the pharmaceutical analyst – partially driven by the heavily regulated nature of the industry – and the dominance of reversed-phase methods make this unlikely.

See also: **II / Chromatography : Liquid :** Column Technology; Ion Pair Liquid Chromatography; Mechanisms: Ion Chromatography; Mechanisms; Normal Phase; Mechanisms: Reversed Phases. **III / Porous Polymers : Liquid Chromatography**.

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Capillary Electrophoresis

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Introduction

In recent years the analysis of pharmaceuticals has been predominantly performed by high performance liquid chromatography (HPLC), which offers a number of advantages over other alternative techniques. These advantages include automated and precise sample injection devices, sensitive detection and high capacity autosamplers. HPLC is supported by other techniques such as thin-layer chromatography (TLC) and gas chromatography (GC). In the late 1980s a further technique, that of capillary electrophoresis (CE), became recognized as a viable alternative and complementary technique to HPLC. Modern CE instruments offer some of the same features as HPLC in terms of automation and autosampler capacity, although precision and sensitivity are not as good.

The wide range of application areas for CE within pharmaceutical analysis mirror well those of HPLC. These areas include the determination of drug-related impurities, chiral separations, main peak assay, stoichiometric determinations and the analysis of vitamins. The majority of pharmaceuticals are synthetic organic molecules that are well suited to analysis by HPLC or CE. There is an increasing move in pharmaceutical companies to the development of new pharmaceuticals that are based on biomolecules such as peptides and DNA. Traditionally these biomolecules have been analysed using electrophoretic techniques; for this reason CE has been widely applied to the analysis of biomolecule pharmaceuticals.

One of the attractive features of CE is that method development can be relatively simple for uncomplicated separations of ionizable pharmaceuticals. For example the majority of pharmaceuticals are basic drug salts. Use of a low pH electrolyte causes these basic drugs to protonate and become cations, and thus allows separation by CE. There are also a number of drugs with acidic functionalities that can